ELSEVIER

Genetically manipulated adult stem cells for wound healing

Li-Hua Peng[1,2], Kwok-Pui Fung[2], Ping-Chung Leung[3] and Jian-Qing Gao[1]

[1] Institute of Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, PR China
[2] Department of Biochemistry, School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, PR China
[3] Department of Orthopaedics and Traumatology, School of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, PR China

New knowledge of the signal controls and activities of adult stem cells (ASCs) involved in wound repair have led to extensive investigation of the topical delivery of biomacromolecules and multipotent stem cells to injured tissues for scar-less regeneration. The transplantation of genetically recombinant stem cells, which have roles as both therapeutics and carriers for gene delivery to wound sites, represents an attractive strategy for wound treatment. Here, we compare viral and non-viral vectors and three-dimensional scaffold-based transfection strategies in terms of their biosafety, recombinant efficiency and influence on the differentiation of ASCs, to indicate the future direction of the application of recombinant ASCs in wound treatment.

Introduction

The loss of integrity of large portions of the skin as a result of injury can lead to major disability or even death. Advances in understanding the molecular and cellular responses involved in wound repair and regeneration has led to the extensive use of cytokine supplements in wound care [1]. However, clinical effects of the application of growth factors to accelerate wound healing have been discouraging and the clinical protocol relating to the optimized profile of the bioactive molecules seems an impossible target to achieve; such a treatment regimen has also suffered from the inherent loss of drug activity owing to the combined effects of physical inhibition and biological degradation of the cytokines. Up until the development of gene transfer technology, the molecular approach, in which genetically modified cells synthesized and delivered the encoding growth factors, transcriptional factors and therapeutic DNAs and/or miRNAs in a time-regulated and locally restricted manner to the wound site, was shown to be a promising way of overcoming the limitations associated with the traditional application of recombinant proteins [2]. In particular, stem cell biology seems to present more opportunities for wound therapy. The growth potential and pluripotency of stem cells make them useful not only as therapeutics, but also as vehicles for gene delivery to the site of injury [3]. As we discuss here, one exciting approach to wound therapy over the coming decades is likely to be the development of recombinant stem cell-based skin grafts that have the capacity to deliver the required growth factors to the injury site and that are then able to develop into new skin layers with normal functions [4,5].

Gene therapy for wound healing

Therapeutic molecules involved in wound healing

The wound healing process in adults can be divided into three overlapping phases: inammation, proliferation and remodeling. These processes involve the coordinated efforts of keratinocytes, fibroblasts, endothelial cells, macrophages and platelets, regulated by a complex signaling network of molecules. The influence and underlying mechanism of these cells and molecules on wound healing has recently been reviewed elsewhere [6–8]; therefore, we only provide here a list of molecules that are currently known to regulate significantly the wound healing outcome (Table 1). How these bioactive molecules can be manipulated by therapeutic intervention represents a key question for the strategy of gene- and cell-based wound therapy. Currently, multiple novel delivery systems (e.g. slow-releasing polymer-based drug delivery systems and viral or nonviral vector-based plasmid DNA transfer systems) have been widely used to deliver growth factors or cytokine genes

Corresponding author:. Gao, J.-Q. (gaojianqing@zju.edu.cn)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2011.07.009

TABLE 1

Therapeutic molecules and their activities in wound repair

Molecules[a]	Reported functions at the wound site			
	Epithelialization	**Inflammation**	**Angiogenesis**	**Matrix deposition**
EGF family				
EGF	+			
HB-EGF	+		+	
FGF-2	+	+		+
FGF-7	+		+	
FGF-10	+			
TGF family				
TGF-β1	+	+		+
TGF-β2	+	+		+
TGF-β3	+	+		
BMP-6	+/−			
Activin	+	+		
VEGF family				
VEGF-A			+	
VEGF-C		+	+	
PLGF				
Other growth factors				
PDGF[b]	+	+	+	+
CTGF	+	+	+	+
GM-CSF[c]	+		+	
NGF	+	+	+	
IGFs	+			+
Cyr61		+	+	+
Cytokines				
IL-1	+	+		+
IL-4	+			+
IL-6	+	+		+
IL-8		+		+
IL-10		−		
TNF-α	+	+		+
Chemokines				
CXCL-1		+	+	
CXCL-5		+	+	+
CXCL-8	+	+		
CXCL-10	−	+		
CXCL-12	+	+	+	
Transcription factors				
C-fos	+			
C-Jun	+			
JunB	−	−		
PPAR β/δ	+			
PPAR-α		−		
E2F-1	+	+		
C-Myb		+		+
C-Myc	+/−			
Stat3	+			
PU1		+		
Nrf2		+		
HIF-1α	+		+	
HOXA3	+		+	
miRNAs				
HOXD3			+	+
CARP			+	
Egr-1	+		+	+
Smad3	−/+	+		+
Smad2	−			
HOXB13		−	+	
β-Catenin	−	+		+
aHIF	+		+	

TABLE 1 (*Continued*)

Molecules[a]	Reported functions at the wound site			
	Epithelialization	Inflammation	Angiogenesis	Matrix deposition
Other molecules				
Basonuclin	+			
Connexin 43		+		
Thymosin β4	+	−	+	+
Fibronectin	+	+	+	+
TRAP		+	+	
IP-10	−		−	
MCP-1	+	+	+	+

[a] ***Abbreviations***: aHIF, antisense-hypoxia inducible factor; CARP, cardiac ankyrin repeat protein; CTGF, connective tissue growth factor; CXCL, C-X-C motif ligand; Cyr61, cysteine-rich 61; Egr-1, early growth response transcription factor-1; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIF-1α, hypoxia-inducible factor 1, alpha subunit; HOXA3, Homeobox protein Hox-A3; HOXB13, Homeobox protein Hox-B13; HOXD3, Homeobox protein Hox-D3; IP-10, interferon-inducible protein-10; MCP-1, monocyte chemotactic protein-1; NGF, nerve growth factor; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PLGF, placenta growth factor; PPAR, peroxisome proliferator-activated receptor; Smad, Drosophila mothers against decapentaplegic protein; Stat3, signal transducer and activator of transcription 3; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha; TRAP, thrombin receptor activating peptide. +, upregulation; −, downregulation; blank cells indicate no apparent influence.
[b] Licensed for the treatment of neuropathic diabetic foot ulcers.
[c] Pilot studies in infected diabetic foot ulcers encouraging.

to the target wound tissues to accelerate repair and improve the quality of healing, respectively. However, the bioactivity of most growth factors and cytokines can be easily affected, owing to the combined effects of physical inhibition and biological degradation; therefore, until recently, clinical trials of the direct topical application of the growth factors reported only limited success. A molecular approach in which genetically modified cells synthesize and deliver the desired growth factor in a regulated fashion has therefore been investigated as an alternative method to overcome the limitations associated with the topical application of recombinant growth factor proteins.

Strategies for the delivery of genes to a wound site

Currently, viral and non-viral vectors, as well as cell-based approaches, have been used for the delivery of wound molecular effectors, each with their own advantages and disadvantages. A viral-based approach is based on the generation of a replication-defective particle by replacing some or all of the viral genes with the gene(s) of interest. Viral vectors can provide high transfection efficiency and rapid transcription of the foreign material into the viral genome. However, many clinical trials of viral vectors have been interrupted, because their application resulted in unexpected adverse effects, including mutagenesis, carcinogenesis and immune responses [9]. After the death of a patient in a clinical trial of viral gene transfer in 1999, non-viral methods, including the delivery of DNA via physical and chemical means, were developed [10]. For example, Hengge *et al*. were the first to develop direct injection of DNA, in this instance encoding the interleukin-8 gene: injecting naked genes into the skin resulted in significant recruitment of dermal neutrophils [11]. Eriksson *et al*. modified the direct injection technique and developed the 'micro-seeding' technique, delivering naked DNA into target cells via solid needles. However, although microinjection was the first direct method of transferring DNA into the cell cytoplasm or nucleus, it does so with low efficacy and is therefore inappropriate for the studies that require a large number of transfected cells [12]. Hydrodynamic injection was investigated widely as a method for *in vivo* targeting, whereby naked nucleic acids are injected in large volume under high pressure into the tail vein of animal models. This technique has proved to be efficient for plasmid DNA (pDNA) and small interfering (si)RNA-targeted delivery to skeletal muscles. However, the nonspecific nature of the technique limits its application in clinical practice [13].

Neumann was the first to demonstrate that DNA could be introduced into living cells by means of electric pulses. This technique was known as electroporation and has been routinely used *in vitro* and *in vivo* in animals [14]. However, the disadvantages of this approach include cell damage and the limited accessibility of the internal organs [15]. A gene gun has been used since 1987 for gene delivery into plant cells; it is a technique that penetrates the cellular membrane through which the gold or tungsten-coated particles carrying DNA plasmids are then propelled [16]. However, the tissue damage accompanying the use of the gene gun in some application is unavoidable and the gene expression is often only found in cells near the exterior surface of the target area [17]. Until recently, most of these physical equipment-based transfection strategies were too inefficient for clinical use (i.e. electroporation and laborious microinjection of DNA).

Compared with the viral and physical transfection techniques, nonviral vectors (e.g. cationic liposomes or polymers) were shown to be safe and flexible, with no additional inflammation and infection risk. The transient gene expression induced by nonviral vectors is an additional advantage for wound treatment, as permanent gene expression is not needed once the wound heals. However, direct gene transfer using nonviral vectors does not enable sophisticated control of the therapeutic genes, which thus limits its use when the targeted organ or tissue is not easily accessible [10]. Living cells as gene-delivery vehicles have therefore received much attention for use in gene therapy. This molecular genetic approach introduces plasmid DNA or genes encoding the required signaling factors into the cells that then act as gene delivery vehicles. Required functional mediators can be expressed and secreted spatially and temporally by the transfected cells. This cellular-based gene delivery is advantageous in that: (i) cells can be manipulated more precisely than in the body; (ii) some of the cell types that continue to divide under laboratory conditions can propagate significantly before reintroduction into the patient; (iii) cells are able to localize to particular regions of the human body and would therefore be useful for applying the therapeutic gene with regional specificity. For wound therapy, cell as candidates for

genetic engineering and gene delivery vehicles can be selected based on their availability, expansion capacity *in vitro*, survival ability after transplantation and ability to differentiation at the injury site [18].

Rationale of using adult stem cells as vehicles for gene delivery in wound therapy

Stem cells are cells that have clonogenic and self-renewing capabilities and that can differentiate into multiple cell phenotypes. They can be classified in various ways; for example, one approach is to identify the source and, thus, stem cells can be regarded as either embryonic or adult in nature. Embryonic stem cells are derived from mammalian embryos during the blastocyst stage and have the ability to generate any terminally differentiated cell in the body [19]. However, despite their potential, there are ethical concerns and issues relating to their use. By contrast, adult stem cells (ASCs) can be harvested directly from the patients themselves and the ethical barrier for clinical application is low; thus, increasing numbers of clinical experiments of cell-based tissue regeneration have been conducted using ASCs. Recently, various ASCs have been identified for their potential as therapeutics for wound treatment. For example, keratinocytes that re-epithelialize wounds have been found to be derived from two populations of epithelial stem cells (epiSC) in the skin: the interfollicular epiSC located in the basal layer of the interfollicular epidermis [20], and the hair follicle (HF) bulge epiSC, located in the outer root sheath of the permanent portion of the HF [21]. They are self-renewing and able to produce transient amplifying cells that undergo several cell divisions before differentiating and leaving the basal compartment. It has been demonstrated that the obvious source for the leucocytes that migrate to the wound site during the early, inflammatory phase are bone marrow (BM)-derived hematopoietic stem cells and BM–mesenchymal stem cells [22]. Currently, the major characteristics and functions of ASCs (including those derived from BM, epidermis, HF and adipose) in wound healing have been identified as: (i) capacity for site-specific differentiation into multiple lineages when expanded to colonies; (ii) high migration potential and appearance in increasing numbers at the wound site; (iii) unlimited expansion with a stable phenotype and able to remain as a monolayer *in vitro*; (iv) easy availability, using less invasive methods; (v) immunosuppressive effects and low mutation rates; (vi) less neurotoxicity and tumorigenicity [23–26]; and (vii) capacity to secrete one or several effectors of epidermal growth factors (EGFs), keratinocyte growth factors, insulin-like growth factor 1 (IGF-1), vascular endothelial growth factors (VEGFs), erythropoietin, stromal cell-derived factor-1, interleukin (IL)-6, IL-8, chemoattractants, macrophage inflammatory proteins, and so on [27,28]. These properties make ASCs not only a cheap and accessible therapeutic resource for treating an increasing number of patients with wounds, but also a promising vehicle for gene delivery to wound sites.

Genetic manipulation of stem cells

There are three main steps to using ASCs both as a source of therapeutics and a gene transfer carrier. First, ASCs are isolated and expanded *in vitro*. Second, the therapeutic gene is transduced into the ASCs, using methods similar to those used in direct gene transfer. Finally, the genetically modified ASCs are injected into the patient. ASC gene transduction is perhaps the key step determining the efficiency of this regenerative therapy. The central issue surrounding the development of ASCs as carriers for gene delivery, is the specific methodology used to introduce therapeutic genes into the cells. In recent years, various carriers of viral and nonviral systems have been developed for stem cell genetic manipulation, and these are reviewed below.

Viral vectors

Because of their high efficiency of transfection, genetically modified viruses have been widely used for the delivery of genes into ASCs. Among them, recombinant retroviruses are the most commonly used vehicles. Lentivirus, adenovirus and nonviral gene transfer technologies have also been used but to a lesser extent [9].

Retroviruses

Retroviruses are capable of simultaneously transferring genes to large numbers of cells with high efficiency. Retroviral modification of BM-derived stem cells (BMSCs) incorporating a high-expression porcine (HEP)-fVIII transgene was reported recently. In this study, BMSCs were transduced *ex vivo* with retroviral vectors and the genetically modified BMSCs were found to secrete high levels of HEP-fVIII into the conditioned medium [29]. Another study reported a promising way of transducing epidermal stem cells (ESCs) in cultures containing both stem and differentiated cells, in which high levels of retroviral gene transfer to human ESCs was obtained by immobilizing the retrovirus on a recombinant fibronectin fragment. Notably, transduction on recombinant fibronectin does not require the addition of toxic polycations, such as polybrene, thus providing a simple, fast and efficient means of modifying human ESCs for cutaneous gene therapy and for biological studies that require stable genetic modification [30]. One obvious limitation of retroviral-mediated gene transfer is that only gene of limited sizes (<6 kbp) can be packaged and transferred [9].

Adenoviruses

Adenoviruses can be easily concentrated to high viral titers and can infect dividing as well as nondividing cells with high efficiency. This is a major advantage in situations where cell division is infrequent, such as for gene transfer to the lung [9]. In a recent study, adipose-derived stem cells (ADSCs) transduced with adenoviral bone morphogenetic protein 4 (Ad-BMP4) were injected directly into the hind limb muscles of athymic mice. Results, in terms of the expression of the osteoblastic gene, alkaline phosphatase (ALP) activity and von Kossa staining, showed that ADSCs transduced with Ad-BMP4 underwent rapid and marked osteoblast differentiation, whereas ADSCs transduced with Ad-enhanced GFP (eGFP), and control cells displayed no osteogenic differentiation. X-ray and histological examination confirmed new bone formation in athymic mice transplanted with ADSCs transduced with Ad-BMP4. This study demonstrated the successful osteogenic differentiation of ADSCs transduced with Ad-BMP4 *in vitro* and *in vivo* [31]. The major disadvantage of the use of adenoviral vectors has been the cytotoxicity of viral proteins and the host cellular immune responses to the adenoviral proteins of the virus particle, which result in local inflammation and destruction of transduced cells. The host immune response also limits repeated administrations of the vector

Adeno-associated viruses

Although adeno-associated viruses (AAV) can transduce a range of dividing and nondividing cells, its main advantage is that the wild-type virus integrates reliable into a specific position on the chromosome, which reduces the probability of inadvertent activation of a proto-oncogene. In AAV vectors that have had the viral recombination function deleted, integration in the host genome occurs randomly as sites of double-stranded (ds)-DNA breaks. Of the various self-complementary AAVs (scAAVs), scAAV2 and scAAV5 effectively and safely expressed transgenes in human mesenchymal stem cells (hMSCs). Transduction efficiency with scAAV2 at 1000 multiplicity of infection in bone marrow and umbilical cord blood derived MSCs was $66.3 \pm 9.4\%$ and $67.6 \pm 6.7\%$, respectively [32]. The disadvantages of AAV vectors are that it is possible to package transgenes only up to 4.7 kbp and recombinant vector preparations are frequently contaminated with wild-type adenovirus, which must be separated or inactivated. Furthermore, the integration mechanism is not as precise as that for retroviral vectors and generally results in tandem repeats of the transgene being inserted into the host chromosome. Immune responses can occur with AAV administration, and many individuals have prior immunity [33].

Lentiviruses

It was recently reported that transcriptionally targeted lentiviral vectors efficiently transduced clonogenic stem cells derived from a skin biopsy of a patient with junctional epidermolysis bullosa, restored normal synthesis of laminin-5 in cultured keratinocytes and reconstituted normal adhesion properties in human skin equivalents transplanted onto immunodeficient mice [34]. In a recent study, a lentivirus vector encoding human hepatocyte growth factor (HGF) was constructed and infected into ADSCs. Results indicated that transplantation of ADSCs transfected by lentiviral vectors was a useful treatment for patients with ischemic heart disease [35].

Non-viral vectors

Viruses are still the main vectors used in clinical trials, providing high transfection efficiency and rapid transcription of the foreign material inserted into the viral genome. However, many clinical trials in which viral vectors were applied have been interrupted because the application of several viruses can integrate stably into the host genome, resulting in stable and long-term gene expression and adverse effects, such as mutagenesis, carcinogenesis and an immune response [30]. By contrast, the nonviral system, in which the plasmid DNA is complexed with cationized carriers, is safer and has no limitation in terms of the molecular size of DNA that can be applied. Increasing numbers of studies have demonstrated that nonvirally induced therapeutic gene expression is a preferable way to adapt stem cells for wound therapy with clinical acceptance [36].

Currently, cationic liposomes and polymers are nonviral vectors that have received significant attention as reliable and efficient vehicles for gene delivery. Lipoplex or polyplex particles are formed from the combination of cationic liposomes or polymers with nucleic acids in buffered, aqueous solution. The particles bind to the cell surface by nonspecific, electrostatic interactions between the positively charged complexes and the negatively charged cell surface, and enter cells by endocytosis or endocytosis-like mechanisms. Once inside, the pH of the endosome compartments drops from pH 7 to 5.5 and part of the bound nucleic acid escapes from early endosomes into the cytosol. However, this mechanism of escape is fundamentally different in lipoplexes compared with polyplexes. Cytoplasmic transport of endosomes has an active role in bringing the transfecting material close to the perinuclear region. The lipoplexes or polyplexes then dissociate and the plasmid DNA released enters the nucleus by one of two hypothesized pathways: (i) passive DNA entry into the nucleus during cell division when the nuclear membrane disintegrates temporarily; or (ii) active transport of the DNA through nuclear pores. Neither mechanism is mutually exclusive in a given cell type. However, nuclear uptake has been demonstrated to be a significant barrier to gene delivery [37].

Cationic liposome- and/or micelle-based carriers

Liposomes and/or micelles are spherical lipid bilayers of diameters in the range of 50–1000 nm that can target many types of drug as well as genes to HFs. Cationic liposomes are synthetically prepared vesicles with positively charged surfaces that form loose complexes with negatively charged DNA to protect it from degradation in the wound environment. Complexing with the cationized liposome converts the charge of the plasmid DNA to positive, enabling interaction with the negative charge of the cell membrane and resulting in enhanced gene transfection into the cells. The liposome can be prepared easily to include large-sized DNA [38] and has been applied to *in vitro* gene transfection. However, the liposome is not always suitable for *in vivo* gene delivery, because liposomes injected intravenously tend to accumulate mainly in the lung [39]. To overcome this issue, modification of the liposome with cell-selective moieties has been attempted experimentally [40]. A variety of stimuli, such as pH, temperature, ultrasonic waves, magnetic fields and light, are currently being investigated for improved liposome- and/or micelle-based gene delivery in various settings [41–44]. Co-application of liposomes with other polymers, such as polyethyleneimine, provides an avenue for improved transfection efficiency [45].

Cationic polymer-based gene carriers

Cationic polymers are amphiphilic molecules containing a positively charged polar headgroup linked to a hydrophobic domain via a connector. They can be combined with DNA to form a particulate complex (a polyplex) that is capable of transferring genes into targeted cells. The first-generation cationic polymers, such as polylysine or polyarginine, were relatively inefficient in terms of endosomal escape and transfection efficiencies. By contrast the second-generation cationic polymers, such as polyethylenimine and polyamidoamine dendrimers (PAMAM), can mediate endosome disruption by acting as proton sponges. Poly(-ethylenimine) (PEI) is one of the most widely studied polymers for gene delivery in the recent years [46].

Nondegradable polymeric vectors

There are several examples of nondegradable polymeric vectors.

(i) Poly(L-lysine) (PLL) is a well-known polycation and has been used to condense plasmid DNA under various salt conditions. PLL has a sufficient number of primary amines with positive charges to interact with the negatively charged phosphate groups of DNA. One popular modification for

increasing both the transfection ability and the circulation half-life of these vectors is coating with poly(ethylene glycol) (PEG), conjugating with chitosan or with lipids. For example, the transfection efficiency of PEG-PLL/pDNA is approximately 30-fold higher than that of PLL/pDNA in tumor cells, despite that the mechanism of transfection enhancement is not clear [47].

(ii) Polyamidoamine dendrimers have a branched spherical shape and a high surface charge density. Their ability to transfect cells depends on the size, shape and number of primary amine groups on the polymer surface and has been used in gene delivery. PAMAM are the two dendrimers that have received the most attention for use in gene delivery. The use of polyamidoamine [PAMAM-NH(2)] dendrimers along with other nonviral vehicles for the *in vitro* transfection of human BMSCs (hBMSCs) and for engineering BMSCs to secrete brain-derived neurotrophic factor has recently been reported [48]. In addition, a new type of cationic lipid, in which the hydrophobic and hydrophilic moieties coexist on opposite sides, results in vector with a hydrophilic interior and a hydrophobic corona. These new vectors show a remarkable capacity for mediating the internalization of pDNA with minimum cytotoxicity. Gene expression in BMSCs with relevancy in the regenerative medicine clinical context is also enhanced [49]. However, the toxicity of dendrimers is of major concern for their medical use. Surface modification with PEG or replacement with low-generation dendrimers have been reported to improve the biocompatibility of these biomaterials [50].

(iii) Poly(ethylene-imine) (PEI) has been revealed to be the most effective nonviral vector based on its favorable characteristics of DNA protection, cell binding and uptake, endosomal escape and release from the carrier. Ahn *et al*. investigated PEI as a gene carrier for human ADSCs (hADSCs). Flow cytometry revealed that the transfection efficiency using PEI at N:P charge ratios of 4 and 8 was higher than that of lipofectamine. The highest transfection efficiency (19%) was obtained at an N:P charge ratio of 8. After transfection, the eGFP started to localize in the nuclei of hADSCs at 4.3 hours and throughout the cytoplasm from 9.3 hours [51]. PEI of a certain high molecular weight is necessary for the efficient delivery of DNA; however, a high molecular weight PEI is cytotoxic and its long-term safety is problematic because of its nonbiodegradability. Thus, to increase the transfection efficiency of PEI-based polyplexes and to reduce its cytotoxicity, various strategies have been investigated. PEI (25 kDa) has been linked with less toxic, biocompatible drug carriers, such as β-cyclodextrin, polyethylene glycol and chitosan. The cytotoxicity of these conjugations was reduced significantly to 0–60%, in comparison with 85–100% for nonmodified PEI. The transfection efficiency of these conjugations was enhanced by 2.5–3.5-fold to PEI or 1000-fold for chitosan alone to BMSCs [51–53].

PEI, dendrimers (pDMAEMA) and PLL are still the most frequently used polymers for gene delivery. These materials are, however, non-degradable and there is consequently a risk that they will accumulate in the body. Most of these high molecular weight cationic polymers are cytotoxic as a result of adverse interactions with membranes, which result in the loss of cytoplasmic proteins and collapse of the membrane potential. By contrast, low molecular weight polymers can exhibit only low colloidal stability and will induce marginal transfection properties owing to their reduced number of electrostatic interactions. Consequently, there is a need for biodegradable gene delivery polymers that have reduced toxicity and do not accumulate in cells. Moreover, the degradation of the polymer can be used as a tool to release the plasmid DNA into the cytosol. Examples of such biodegradable gene delivery products are detailed below.

(i) Protamine sulfate is smaller than PLL, and highly positively charged owing to the presence of 21 arginine residues. Although the positive charge on its amino groups facilitates charge interactions with the phosphate backbone of DNA, it is the multivalent nature of the polyamines that confers their ability to condense plasmid DNA, and provide complete protection of DNA from nuclease degradation. It has been hypothesized that the sequence of the cationic polypeptide of protamine mimics nuclear localization signals that might facilitate nuclear uptake and makes it a transfection accelerator [54].

(ii) Chitosans of 10–50 kDa are excellent gene transfer reagents. The optimum molecular weight of chitosan is dependent on the cell lines. However, the stability of the complex in serum decreases when the molecular weight of chitosan is less than 5000 Dalton. Chitosan derivatives, such as deoxycholic acid modified-chitosan, dodecylated chitosan and quaternized chitosan, have also been reported to be effective vectors for gene transfer [55]. Recently, the cytotoxicity and transfection efficiency of chitosan–DNA complexes combined with PEI was investigated [53]. The combination of PEI with chitosan–DNA complexes was shown to enhance gene expression markedly to 1000-fold of that induced by chitosan alone; in addition, the cytotoxicity of the PEI decreased considerably upon combination with the chitosan–DNA complexes [53].

(iii) Cationized polysaccharides, such as cationized dextran [56] and schizophyllan [57], are several novel nonviral vectors reported in recent years. Advantages of these polysaccharide-based carriers over other cationized polymers include the presence of hydroxyl groups available for simple chemical modification and the cell internalization possibly accelerated by a sugar-recognition receptor on the cell surface. For example, complexation with the spermine-dextran or the spermine-pullulan [58] vector enabled the plasmid DNA to decrease the apparent size of the vector–DNA complex until it was small enough to internalize into cells by way of a sugar-recognizable receptor and to enhance the expression level of plasmid DNA [59]. These are thought to be promising vectors, with the added benefit of biodegradability, for enhancing the gene expression of MSCs and dendritic cells through receptor-mediated endocytosis, with wide potential for use in tissue regeneration [60,61].

Scaffold-based stem cell recombination

Currently, the system that combines a nonviral gene carrier and the three-dimensional (3D) scaffold in which the stem cells are transfected with the genes released from, or substrated to, the 3D

scaffold represents an attractive strategy for enhanced stem cell recombination in tissue engineering [62]. The rationale of using this strategy is attributed to the 3D scaffolds, which can provide a larger surface area for cell attachment, spreading, proliferation and adhesion than that available in two-dimensional culture plates, and an environment with the persistent release of target genes for the multiple transfection of cells. Additionally, the 3D-scaffold surface controls the spatial arrangement of cells and their transmission of biochemical and mechanical signals that govern gene expression [63]. It is a strategy that combines the most up-to-date methods of gene delivery and cell transplantation, in which genetic recombinant stem cells act both as seed cells for tissue engineering and as the vehicle for gene delivery needed for the wound repair and regeneration [64] (Fig. 1). Techniques used in

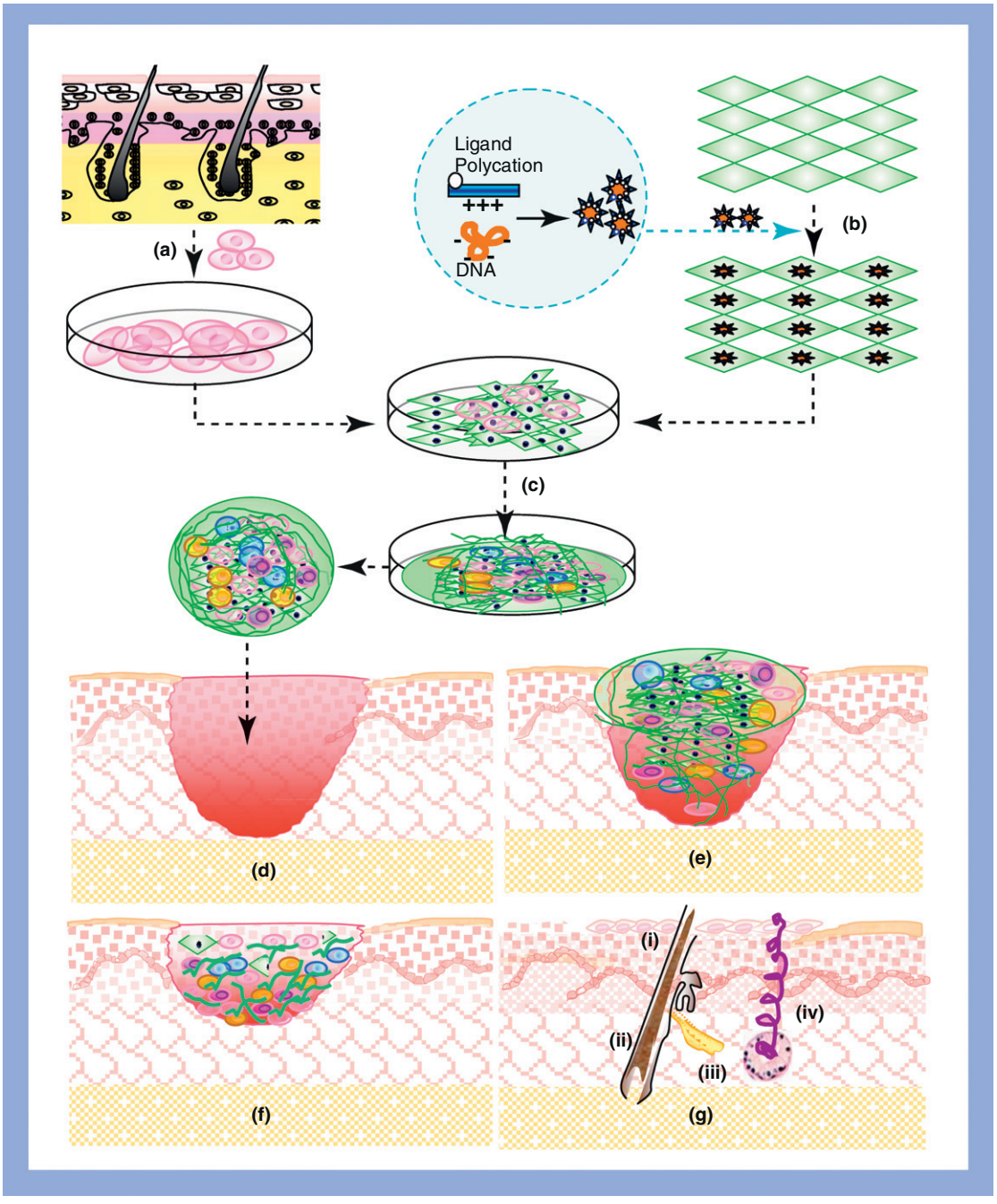

FIGURE 1

Schematic diagram of the construction of recombinant stem cell-based skin substitute and its application in wound healing. **(a)** Isolation and culture of adult stem cells. **(b)** Construction of the three-dimensional (3D) scaffold with the gene–vector complex incorporated. **(c)** Co-culture of the stem cells with the functional scaffold (cell propagation and gene transfection involved). **(d)** Application of recombinant stem cells and 3D scaffold-based skin substitute to the wound site. **(e)** Degradation of the scaffold and release of the recombinant stem cells in the topical area. **(f)** Growth, differentiation and adhesion of the stem cells and the expression of the functional genes in the injury site. **(g)** Regeneration of the skin layers and appendages [(i) epidermal and dermal layers; (ii) hair follicle; (iii) sebaceous gland; (iv) sweat gland].

the incorporation of genes within polymeric scaffolds can be divided into entrapment (polymeric release) and attachment (substrate mediated means) approaches. In the polymeric release approach, the DNA complexed with cationic lipids or cationic polymers is incorporated and released from the scaffold while maintaining its activity. Substrate-mediated DNA delivery is based on the immobilization of complexes within, or to, a biomaterial by drying, nonspecific adsorption and biotin/avidin binding, which serves as a substrate for cell adhesion [65]. Yang Fan *et al*. reported that the implantation of scaffolds seeded with VEGF-expressing stem cells led to two to fourfold-higher vessel densities 2 weeks after implantation, compared with control cells or cells transfected with VEGF by using Lipofectamine2000. The genetically modified hBMSCs substantially enhanced angiogenesis and limb salvage, while reducing muscle degeneration and tissue fibrosis after 4 weeks [66]. The recent incorporation of a gene into electrospun nanofibers for localized gene transfection of target cells has also resulted in increased interest in tissue engineering. By encapsulating target genes within the scaffold during the fiber drawing process, the controlled release of genes over time could be achieved, while retaining their bioactivity, and successfully seeded transfected cells onto the scaffold in a sustained manner under physiological conditions [67–70]. For example, PEG-modified PEI was incorporated into a scaffold by electrospinning and target DNA was then adsorbed onto the electrospun nanofibers via electrostatic interactions between DNA and PEI–PEG. This nanofiber-based gene delivery system exhibited high transfection efficiency, in which >40% of mesenchymal stem cells were transfected. All these results demonstrate that the combination of nonviral gene carriers with electrospun nanofibers could be used for localized gene delivery, which has promising application as a functional substrate for skin regeneration.

Clinical outlook

Based on the increasing number of results highlighting the potential role of ASCs in wound healing, their application is believed to be advantageous over that of the administration of single biological diffusible factors, because ASCs can interact with their environment and release multiple wound healing factors. However, several issues need to be considered before their clinical application, including: (i) the suitability of the patient suitable for stem cell treatment and which would be the most appropriate stem cell population. Patient characteristics, including their age, should be taking into consideration, because the functionality of stem cells decreases with age, even if the quantity does not. The autograft of stem cells in this situation should therefore be avoided [71]; (ii) different stem cell types and application strategies should be selected based upon the pathology of the wound. For example, although hMSCs might help reduce scarring and restore tensile strength in acute wounds [72], patients predisposed to keloid disease should avoid BMMSC application to their wounds. So far, these keloid-promoting effects have been reported from BMSCs, although the same effects from transplanting other ASC, such as those from the skin, HF and adipose tissue, have not. The role of these cutaneous stem cells in the pathogenesis of wound-healing disorders therefore requires more investigation before their clinical application. (iii) Despite the fact that BMMSCs are able to interact with dendritic cells, T cells from the host promote a more anti-inflammatory and tolerant phenotype [73]. The possibility of immunorejection that might be triggered by the transplantation of other ASCs therefore needs more investigation. (iv) Stem cells should ideally be able to retain their 'stemness' until they are delivered to, and maintained, at the wound site, to enhance engraftment [74]. In such cases, 3D scaffolds with or without genes incorporated are thought to be the superior carrier for delivering stem cells to the wounds locally, which also prevents the systemic distribution of the cells. Thus, this is likely to be a more effective method for targeting the wound site compared with injection. This delivery technology is a blend of gene therapy and tissue engineering in that genes are formulated with porous biomaterial scaffolds, an environment of guided tissue regeneration. The scaffold fills the wound bed, holding the DNA vector *in situ* until endogenous repair cells arrive. These cells migrate along the scaffold and, once transfected, essentially become local *in vivo* bioreactors that produce the therapeutic factors encoded by the DNA. Thus, gene delivery and expression are local rather than systemic phenomena, and cell targeting is a passive rather than an active process. Typically, the therapeutic goal of wound healing is to induce new tissue formation in a nonhealing wound or to inhibit an exaggerated tissue-repair response. Viral vectors maximize gene-transfer efficiency and could be formulated with biomaterial scaffolds. However, unacceptable immune responses that decrease efficacy and increase toxicity limit the clinical use of viral vectors. Increasing amounts of research has focused on nonviral vectors encoded with plasmid DNA as a delivery system. However, for tissue regeneration, large amounts of DNA have been required in preclinical animal models, and high-dose administration in humans might be limited. In this regard, the potency and safety of nonviral vector-based transfection systems both need to be improved before they could be realistically used in human therapy. (v) More insight into the potential for adverse interactions between local tissues and gene-therapy formulations is required. Necropsy and biodistribution studies are the best way to evaluate potential tissue toxicity. It is hard to imagine a gene-therapy product that does not involve regulated therapeutic gene expression as a way to avoid toxicity. To qualify, a system conferring regulated gene expression should feature low baseline transgene expression, a high induction ratio and be tightly controlled. Nonetheless, the relative role and contribution of locally maintained progenitor cells to skin wound healing compared with different type of ASCs upon cutaneous wounding need further elucidation. It is apparent that current data from preclinical studies are not sufficient to show whether the transplanted stem cell-derived skin appendages can result in normal function; the answers will need to come from more randomized clinical trials.

Challenges and future perspectives

Skin wounds, and the clinical strategies to treat them, have changed significantly over the past century. Apart from function restoration, other important results of wound healing now include improved cosmesis and quality of life. In deep, partial and full-thickness skin injuries, substantial areas of skin are damaged, accompanied by the complete destruction of epithelial-regenerative elements and the loss of skin regeneration capacity; deep wounds can heal only by contraction, with epithelialization

limited to the wound edge and frequently result in cosmetic, functional defects. Although cadaver skin, autografts and allografts have been transplanted, none of these approaches are ideal, as they are have a limited supply, are of high cost and are variable in quality, with many additional safety concerns. Significant progress has therefore been made in the development of cultured autologous sheets of keratinocytes, dermal matrix and 'full skin', with skin cells seeded on acellular dermal scaffolds.

However, the use of traditional substitutes composed by skin stem cells in clinical settings has received less than satisfactory results with problems of loose attachment, delays in vascularization, loss of the cultured autograft after the initial graft and, most importantly, the failure to generate any function of the repaired tissue. The topical delivery of ASCs with or without genetic manipulation in wound therapy has been shown a novel strategy with potential to solve the drawbacks of traditional tissue engineering. For example, purified ESCs have been shown an enhanced capacity to regenerate functional human epidermis [75]. The cross-germ plasticity of ADSCs, in terms of their abilities to differentiate into cells both of mesodermal and nonmesodermal origins, as well as to release angiogenic factors, are in agreement with the enhanced healing upon treatment with ADSC-derived matrix [76].

It is interesting that the genetic manipulation of stem cells before transplantation has received increasing attention, with respect to augmenting the supply of deficient hormones and enhancing the directed differentiation of stem cells into specific lineages [77]. It was shown that human platelet-derived growth factor (hPDGF)-A/hBD2 gene BMSCs modified by recombinant adenovirus vectors were locally transplanted onto the injury site, with a better combined effect over the transplantation of BMSCs alone [78]. In another study, the transplantation of ADSCs encoding human lenti-hHGF led to improved tissue repair and ventricular function, explained partly through their enhanced ability to differentiate into endothelial cells, resulting in increased blood flow and decreased fibrosis by the encoded hHGF [79]. Recombinant HFSCs are newly demonstrated to be a potential source of the next-generation skin substitute because of their ability to regenerate into skin composed entirely of HF cells [80]. It was demonstrated that reconstituted HF from the cultured HFSCs harbor the transgene. The transgene was expressed in all reconstituted epithelial skin compartments, including the HF epithelium, sebaceous gland epithelium and the epidermis, for at least six months after transplantation, indicating that the recombinant HFSCs might provide long-standing therapeutic benefits [81]. In addition, genetic modification of the engineered tissue could be used to increase the rate of graft survival by, for example, promoting angiogenesis.

In these recombinant stem cell-based strategies, safe and efficient genetic recombination of the stem cells with the scaffold based transfection system is the key technology to achieve the delivery of therapeutic genes and to induce the regeneration of skin appendages. Accordingly, several challenges have to be met, such as the development of novel methods with clinical safety and therapeutic efficiency for increased gene transfer to the stem cell compartment; biomaterials for efficient and localized gene delivery; and construction of advanced regulatable vectors for temporal and spatial control of gene expression in response to physiological changes in the body. Moreover, as wound healing is regulated by many growth factors, cytokines and enzymes, with most wound signals controlling more than one cell activity, many cellular and molecular activities are responses to multiple signals, which highlights the need to move towards multiple gene delivery systems or to address key control genes regulating the entire cascade of complex processes, using microarray and proteomic technologies, as well as to establish which regulatory factors need to be activated at what time. Despite that the gene transfection of stem cells with the 3D scaffold is significantly enhanced in contrast to the traditional 2D transfection system, the transfection efficacy is still relatively low and not in strict control. Because many cytokines proposed for use in wound healing could have detrimental side effects if overexpressed in nontarget organs, it is fundamental to understand thoroughly the biological requirements for skin regeneration, as well as to construct molecular-scale scaffolds to achieve precise genetic modification of the stem cells for initiating *in vivo* functions with maximum control. Only if these challenges can be met will this promising strategy be used to treat patients.

References

1 Werner, S. and Grose, R. (2002) Regulation of wound healing by growth factors and cytokines. *Physiol Rev.* 83, 835–870
2 Eming, S.A. *et al.* (2007) Gene therapy and wound healing. *Clin. Dermatol.* 25, 79–92
3 Dalberto, T.P. *et al.* (2010) Mesenchymal stem cells as a platform for gene therapy protocols. *Sci. Prog.* 93, 129–140
4 Ozawa, K. *et al.* (2008) Cell and gene therapy using mesenchymal stem cells (MSCs). *J. Autoimmun.* 30, 121–127
5 Porada, C.D. *et al.* (2006) Adult mesenchymal stem cells: a pluripotent population with multiple applications. *Curr. Stem Cell Res. Ther.* 1, 365–369
6 Velnar, T. *et al.* (2009) The wound healing process: an overview of the cellular and molecular mechanisms. *J. Int. Med. Res.* 37, 1528–1542
7 Gill, S.E. and Parks, W.C. (2008) Metalloproteinases and their inhibitors: regulators of wound healing. *Int. J. Biochem. Cell Biol.* 40, 1334–1347
8 Dorne, R. *et al.* (2007) Wound fluids: a window into the wound environment? *Int. J. Lower Extremity Wounds* 6, 262–272
9 Escámez, M.J. *et al.* (2008) Assessment of optimal virus-mediated growth factor gene delivery for human cutaneous wound healing enhancement. *J. Invest. Dermatol.* 128, 1565–1575
10 Niidome, T. *et al.* (2002) Gene therapy progress and prospects: nonviral vectors. *Gene Ther.* 9, 1647–1652
11 Hengge, U.R. *et al.* (1995) Cytokine gene expression in epidermis with biological effects following injection of naked DNA. *Nat. Genet.* 10, 161–166
12 Eriksson, E. *et al.* (1998) In vivo gene transfer to skin and wound by microseeding. *J. Surg. Res.* 78, 85–91
13 Al-Dosari, M.S. *et al.* (2005) Hydrodynamic delivery. *Adv. Genet.* 54, 65–82
14 Neumann, E. *et al.* (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* 1, 841–845
15 Yao, S.M. *et al.* (2009) Improvement of electroporation to deliver plasmid DNA into dental follicle cells. *Biotechnol. J.* 4, 1488–1496
16 Klein, T.M. *et al.* (1987) High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature* 327, 70–73
17 Kim, D. *et al.* (2008) Enhancement of DNA vaccine potency through coadministration of CIITA DNA with DNA vaccines via gene gun. *J. Immunol.* 180, 7019–7027
18 Johnson, S.M. *et al.* (2001) Gene therapy: optimising DNA delivery to the nucleus. *Curr Drug Targets* 2, 371–399
19 Hadjantonakis, A. and Papaioannou, V. (2001) The stem cells of early embryos. *Differentiation* 68, 159–166
20 Liang, L. and Bickenbach, J. (2002) Somatic epidermal stem cells can produce multiple cell lineages during development. *Stem Cells* 20, 21–31

21 Morris, R. *et al.* (2004) Capturing and profiling adult hair follicle stem cells. *Nat. Biotechnol.* 22, 411–417
22 Li, Y. *et al.* (2008) Human mesenchymal stem cells license adult $CD34_1$ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the notch pathway. *J. Immunol.* 180, 1598–1608
23 Mackenzie, T.C. *et al.* (2001) Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep. *Blood Cells Mol. Dis.* 27, 601–604
24 Pittenger, M.F. *et al.* (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147
25 Saito, T. *et al.* (2002) Xenotransplant cardiac chimera: immune tolerance of adult stem cells. *Ann. Thorac. Surg.* 74, 19–24
26 Chan, J. *et al.* (2005) Human fetal mesenchymal stem cells as vehicles for gene delivery. *Stem Cells* 23, 93–102
27 Waltera, M.N.M. *et al.* (2010) Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an *in vitro* study of fibroblast and keratinocyte scratch assays. *Exp. Cell Res.* 316, 1271–1281
28 Chen, L. *et al.* (2008) Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS ONE* 3, e1886
29 Doering, C.B. (2008) Retroviral modification of mesenchymal stem cells for gene therapy of hemophilia. *Methods Mol. Biol.* 433, 203–212
30 Lei, P. and Andreadis, T. (2008) Efficient retroviral gene transfer to epidermal stem cells. *Methods Mol. Biol.* 433, 367–379
31 Lin, L. *et al.* (2009) Comparison of osteogenic potentials of BMP4 transduced stem cells from autologous bone marrow and fat tissue in a rabbit model of calvarial defects. *Calcif. Tissue Int.* 85, 55–65
32 Kim, S.J. *et al.* (2007) Stable gene expression by self-complementary adeno-associated viruses in human MSCs. *Biochem. Biophys. Res. Commun.* 360, 573–579
33 Murphy, S.L. *et al.* (2009) Diverse IgG subclass responses to adeno-associated virus infection and vector administration. *J. Med. Virol.* 81, 65–74
34 Di-Nunzio, F. *et al.* (2008) Correction of laminin-5 deficiency in human epidermal stem cells by transcriptionally targeted lentiviral vectors. *Mol. Ther.* 16, 1977–1985
35 Morizono, K. *et al.* (2003) Multilineage cells from adipose tissue as gene delivery vehicles. *Hum. Gene Ther.* 14, 59–66
36 Eming, S.A. *et al.* (2004) Gene transfer in tissue repair: status, challenges and future directions. *Expert Opin. Biol. Ther.* 4, 1373–1386
37 Tros de llarduya, C. *et al.* (2010) Gene delivery by lipoplexes and polyplexes. *Eur. J. Pharm. Sci.* 40, 159–170
38 Schmidt-Wolf, G.D. *et al.* (2003) Non-viral and hybrid vectors in human gene therapy: an update. *Trends Mol. Med.* 9, 67–72
39 Kawakami, S. *et al.* (2005) Enhanced gene expression in lung by a stabilized lipoplex using sodium chloride for complex formation. *J. Gene Med.* 7, 1526–1533
40 Sapra, P. *et al.* (2005) Ligand-targeted liposomes for cancer treatment,. *Curr. Drug Deliv.* 2, 369–381
41 Kale, A.A. and Torchilin, V.P. (2007) Enhanced transfection of tumor cells *in vivo* using 'Smart' pH-sensitive TAT-modified pegylated liposomes. *J. Drug Targets* 15, 538–545
42 Leong-Poi, H. *et al.* (2007) Therapeutic arteriogenesis by ultrasound mediated VEGF165 plasmid gene delivery to chronically ischemic skeletal muscle. *Circ. Res.* 101, 295–303
43 Zheng, X. *et al.* (2009) Preparation and characterization of magnetic cationic liposome in gene delivery. *Int. J. Pharm.* 366, 211–217
44 Wu, G. *et al.* (2008) Remotely triggered liposome release by nearinfrared light absorption via hollow gold nanoshells. *J. Am. Chem. Soc.* 130, 8175–8177
45 Hanzlikova, M. *et al.* (2009) The role of PEI structure and size in the PEI/liposome-mediated synergism of gene transfection. *Plasmid* 61, 15–21
46 Yang, F. *et al.* (2009) Gene delivery to human adult and embryonic cell-derived stem cells using biodegradable nanoparticulate polymeric vectors. *Gene Ther.* 16, 533–546
47 Karakoti, A.S. *et al.* (2011) PEGylated inorganic nanoparticles. *Angew. Chem. Int. Ed.* 10.1002/anie.201002969
48 Dai, J. *et al.* (2011) Polyethylenimine-grafted copolymer of poly(L-lysine) and poly(ethylene glycol) for gene delivery. *Biomaterials* 32, 1694–1705
49 Shakhbazau, A. *et al.* (2010) Use of polyamidoamine dendrimers to engineer BDNF-producing human mesenchymal stem cells. *Mol. Biol. Rep.* 37, 2003–2008
50 Santos, J.L. *et al.* (2010) Functionalization of poly(amidoamine) dendrimers with hydrophobic chains for improved gene delivery in mesenchymal stem cells. *J. Control. Rel.* 144, 55–64
51 Tang, G.P. *et al.* (2003) Polyethylene glycol modified polyethylenimine for improved CNS gene transfer: effects of PEGylation extent. *Biomaterials* 24, 2351–2362
52 Tang, G.P. *et al.* (2006) Low molecular weight polyethylenimines linked by beta-cyclodextrin for gene transfer into the nervous system. *J. Gene Med.* 6, 736–744
53 Zhao, Q.Q. *et al.* (2009) N/P ratio significantly influences the transfection efficiency and cytotoxicity of a polyethylenimine/chitosan/DNA complex. *Biol. Pharm. Bull.* 32, 706–710
54 Tsuchiya, Y. *et al.* (2006) Characterization of protamine as a transfection accelerator for gene delivery. *J. Bioact. Compat. Polym.* 21, 519–537
55 Weecharangsan, W. *et al.* (2008) Evaluation of chitosan salts as non-viral gene vectors in CHO-K1 cells. *Int. J. Pharm.* 348, 161–168
56 Jo, J. *et al.* (2010) Preparation of cationized polysaccharides as gene transfection carrier for bone marrow-derived mesenchymal stem cells. *J. Biomater. Sci. Polym. Ed.* 21, 185–204
57 Nagasaki, T. *et al.* (2004) Long-term expression with a cationic polymer derived from a natural polysaccharide: schizophyllan. *Bioconjug. Chem.* 15, 249–259
58 Kanatani, I. *et al.* (2006) Efficient gene transfer by pullulan-spermine occurs through both clathrin- and raft/caveolae-dependent mechanisms. *J. Control. Rel.* 116, 75–82
59 He, C.X. *et al.* (2011) Effective gene delivery to mesenchymal stem cells based on the reverse transfection and three-dimensional cell culture system. *Pharm. Res.* 28, 1577–1590
60 Nagane, K. *et al.* (2010) Promoted adipogenesis of rat mesenchymal stem cells by transfection of small interfering RNA complexed with a cationized dextran. *Tissue Eng. A* 16, 21–31
61 Thakor, D.K. *et al.* (2011) Nontoxic genetic engineering of mesenchymal stem cells using serum-compatible pullulan-spermine/DNA anioplexes. *Tissue Eng. C* 17, 131–144
62 Ahn, H.H. *et al.* (2008) Polyethyleneimine-mediated gene delivery into human adipose derived stem cells. *Biomaterials* 29, 2415–2422
63 Jo, J. *et al.* (2008) Non-viral gene transfection technologies for genetic engineering of stem cells. *Eur. J. Pharm. Biopharm.* 68, 90–104
64 Conrad, C. *et al.* (2007) Genetically engineered stem cells for therapeutic gene delivery. *Curr. Gene Ther.* 7, 249–260
65 Jang, J.H. *et al.* (2004) Gene delivery from polymer scaffolds for tissue engineering. *Exp. Rev. Med. Dev.* 1, 127–138
66 Yang, F. *et al.* (2010) Genetic engineering of human stem cells for enhanced angiogenesis using biodegradable polymeric nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* 107, 3317–3322
67 Zhang, J. *et al.* (2010) Co-electrospun fibrous scaffold-adsorbed DNA for substrate-mediated gene delivery. *J. Biomed. Mater. Res. A* 96, 212–220
68 Ji, W. *et al.* (2011) Bioactive electrospun scaffolds delivering growth factors and genes for tissue engineering applications. *Pharm. Res.* 28, 1259–1272
69 Wan, F. *et al.* (2010) A chemistry/physics pathway with nanofibrous scaffolds for gene delivery. *Phys. Chem. Chem. Phys.* 12, 12379–12389
70 Saraf, A. *et al.* (2009) Regulated non-viral gene delivery from coaxial electrospun fiber mesh scaffolds. *J. Control. Rel.* 143, 95–103
71 Racila, D. and Bickenbach, J.R. (2009) Are epidermal stem cells unique with respect to aging? *Aging* 8, 746–750
72 Stoff, A. *et al.* (2009) Promotion of incisional wound repair by human mesenchymal stem cell transplantation. *Exp. Dermatol.* 18, 362–369
73 Aggarwal, S. and Pittenger, M.F. (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105, 1815–1822
74 Neuss, S. *et al.* (2004) Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. *Stem Cells* 22, 405–414
75 Claudinot, S. *et al.* (2005) Long-term renewal of hair follicles from clonogenic multipotent stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 102, 14677–14682
76 Xie, J.L. *et al.* (2007) A study of using tissue-engineered skin reconstructed by candidate epidermal stem cells to cover the nude mice with full-thickness skin defect. *J. Plast. Reconstr. Aesthet. Surg.* 60, 983–990
77 Thomas-Virnig, C.L. *et al.* (2009) Inhibition of multidrug-resistant *Acinetobacter baumannii* by nonviral expression of hCAP-18 in a bioengineered human skin tissue. *Mol. Ther.* 17, 562–569
78 Hao, L. *et al.* (2009) Transplantation of BMSCs expressing hPDGF-A/hBD2 promotes wound healing in rats with combined radiation-wound injury. *Gene Ther.* 16, 34–42
79 Zhu, X.Y. *et al.* (2009) Transplantation of adipose-derived stem cells overexpressing hHGF into cardiac tissue. *Biochem. Biophys. Res. Commun.* 379, 1084–1090
80 Reynolds, A.J. and Jahoda, C.A.B. (2003) Plasticity of hair follicle dermal cells in wound healing and induction. *Exp. Dermatol.* 12, 126–136
81 Nakagiri, Y.S. *et al.* (2006) Hair follicle stem cell-targeted gene transfer and reconstitution system. *Gene Ther.* 13, 732–737